Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 358-417 are pending in the application, with claims 358 and 411 being the independent claims. Claims 1-309 were previously canceled without prejudice to or disclaimer of the subject matter recited therein. Claims 310-357 are sought to be canceled without prejudice to or disclaimer of the subject matter recited therein. New claims 358-417 are sought to be added.

New claims 358-383 and 387-414 are equivalent to canceled claims 310-357, with the exception that new claims 358 and 411 recite the amount of chloride ion present in the claimed compositions. Support for this can be found in the specification at page 31, paragraph [0081]. The added number of new claims is due to the dividing of the Markush groups in the previously canceled claims. New claims 359-364, 366-367, 369, 371, 375, 377, 380-383, and 387-411 are identical to canceled claims 311-316, 318-319, 321, 323, 325, 326-329, and 330-354, respectively. New claims 365 and 372 are equivalent to canceled claim 317. New claims 368 and 373 are equivalent to canceled claim 320. New claims 370, 374 and 378 are equivalent to canceled claim 322, and new claims 371, 376, and 379 are equivalent to canceled claim 324. New claims 412-414 are equivalent to canceled claims 355-357, respectively, with the exception that these dependent claims have been amended to recite a composition, rather than a method. Canceled claims 355-357 were incorrectly drafted as method claims dependent upon a composition claim (*i.e.*, independent composition claim 354) and have been replaced accordingly by dependent composition

claims 412-414. Replacement claims are presented for the Examiner's convenience and to put the application in better form for allowance. Their entry is respectfully requested.

New claims 384-386 and 415-417 are not equivalent to any of canceled claims 310-357. New claims 384-386 and 415-417 are dependent claims that recite specific molar concentrations of chloride in the aqueous solutions of claims 358 and 411. Support for new claims 384-386 and 415-417 can be found in the specification at page 31, paragraph [0081]. These claims are added to put the application in better form for allowance and their entry is respectfully requested.

New claims 358-362, 380-391, 393, 396-400, and 404-409 read upon the elected species of the invention.

Applicants note that the Examiner states that because claims limited to the species of auxiliary agent, Pluronic® R 25R2, were found to be novel and unobvious over the prior art, "the Office now selects another species of auxiliary agent from the originally available species disclosed in the invention, i.e. Pluronic F63." (Office Action, at page 2, lines 13-18.) Applicants respectfully direct the Examiner's attention to the specification, at pages 35-45, paragraphs [0093] to [0107], which lists various examples of auxiliary agents, and note that "Pluronic F63" is not listed in this section of the specification or at any place within the current specification. Applicants respectfully assert that "Pluronic F63" is a typographical error and that the Examiner intended to specify either Pluronic® L63 or Pluronic® F68 as the second species of auxiliary agent for the purpose of prosecuting the current application. Applicants request that the Examiner inform them whether this assumption is correct, and which species of auxiliary agent the Examiner in fact selected for prosecution.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding rejections and that they be withdrawn.

I. The Rejection of the Claims Under 35 U.S.C. § 112, Second Paragraph

The Examiner rejects claims 310, 311, 313, 314, 326, 328-334, 336, 339-343, 347-349, 351, and 352 under 35 U.S.C.§112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. (Office Action, at page 3.)

Specifically, the Examiner asserts that these claims are indefinite because the metes and bounds of "essentially free of chloride anion" are unclear. (Office Action, at page 3, lines 12-13.)

To expedite prosecution and without acquiescing in the propriety of the rejection, Applicants have canceled claims 310, 311, 313, 314, 326, 328-334, 336, 339-343, 347-349, 351, and 352, thereby rendering moot the Examiner's rejection of these claims. Applicants, however, have added new claim 358, which is identical to canceled claim 310, with the exception that the phrase "essentially free of chloride ion" has been replaced with a specific chloride ion molar concentration range of 0 mM to about 50 mM. New claims 359-410, which depend either directly or indirectly from claim 358, also incorporate this amendment.

Applicants assert that the rejection of claims 310, 311, 313, 314, 326, 328-334, 336, 339-343, 347-349, 351, and 352 under 35 U.S.C.§112, second paragraph, has been overcome and respectfully request the Examiner to reconsider and withdraw this rejection.

II. The Rejection of the Claims under 35 U.S.C. § 103

The Examiner also rejects claims 310, 311, 313, 314, 326, 328-334, 336, 339-343, 347-349, 351, and 352 under 35 U.S.C. § 103(a) as being unpatentable over Liu *et al.* (U.S. Pat. No. 6,120,794) ("Liu") in view of Ulmer, *Science 259*:1745-1749 (1993) ("Ulmer"), Wheeler *et al.* (U.S. Pat. No. 5,861,397) ("Wheeler"), Gregoriadis, *FEBS Lett. 402*:107-110 (1990) ("Gregoriadis"), Ishii *et al.*, *AIDS Res. Hum. Retrovir. 13*:1421-1428 (1997) ("Ishii"), and Hartikka *et al.*, *Gene Therapy 7*:1171-1182 (2000) ("Hartikka"). (Office Action, at page 4.) The Examiner asserts that in light of these documents, "the invention as a whole was *prima facie* obvious." (Office Action, at page 8, line 6.) Applicants respectfully traverse this rejection.

In order to establish a *prima facie* case of obviousness under 35 U.S.C. § 103 in view of a combination of prior art references, the following two conditions must be met: 1) the prior art must suggest to those of ordinary skill that they should perform the claimed method; and 2) the prior art must also provide a reasonable expectation of success. *In re Vaeck*, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991) ("Both the suggestion and the reasonable expectation of success must be found in the prior art, not in the applicant's disclosure."). The prior art references, when combined, must also teach or suggest all the claim limitations.

To expedite prosecution and without acquiescing in the propriety of the rejection, Applicants have canceled claims 310, 311, 313, 314, 326, 328-334, 336, 339-343, 347-349, 351, and 352, thereby rendering moot the Examiner's rejection of these claims.

However, with respect to new claims 358-417, Applicants assert that neither Liu, Ulmer, Wheeler, Gregoriadis, nor Ishii, alone or in combination with one another, would

have provided the required suggestion to perform the claimed invention or a reasonable expectation that the claimed invention would be successful. Neither of these documents, alone or in combination with one another, teach or suggest all the limitations of Applicants' claimed invention as recited in new claims 358-417. Thus, contrary to the Examiner's statements, none of these documents, alone or in combination, would have rendered Applicants' claimed invention *prima facie* obvious.

Applicants note that the Examiner has cited Hartikka as a document that, when combined with Liu, Ulmer, Wheeler, Gregoriadis, and/or Ishii, renders Applicants' claimed invention *prima facie* obvious. Applicants respectfully assert that the use of Hartikka as a reference under 35 U.S.C. § 103(a) is not proper.

Hartikka was published in the July, 2000, issue of Gene Therapy (Volume 7), after the April 21, 2000, filing date of U.S. Appl. No. 60/198,823, the first provisional U.S. application to which the present application claims priority, and less than one year before April 23, 2001 and November 28, 2000, the filing dates of the current application and second priority Appl. No. 60/253,153, respectively. Hartikka cannot qualify as a prior art reference under 35 U.S.C. § 102(a), and thus not under 35 U.S.C. § 103(a), because Hartikka is Applicants' disclosure of their own work. Although Hartikka lists co-authors in addition to Applicants, a rejection under 35 U.S.C. § 102(a) may still be overcome by establishing that the portions of the reference pertinent to the claimed invention describe Applicants' own work. See *In re Katz*, 215 U.S.P.Q. 14 (C.C.P.A. 1982). In their attached Declaration under 37 C.F.R. § 1.132, Applicants Jukka Hartikka, Jennifer Meek, and Marston Manthorpe state that co-authors V. Bozoukova, D. Jones, R. Mahajan, M.K. Wloch, M. Sawdey, C. Buchner, L. Sukhu, K.M. Barnhart, A.M. Abai, and N. Shen performed

experiments disclosed in the Hartikka document that, although supportive of the conclusions reached in Hartikka, do not amount to an inventive contribution to the "salt" element of the claimed invention of the present application.

Thus, in light of the Declaration under 37 C.F.R. § 1.132, Hartikka cannot serve as a prior art reference in a rejection of the pending claims under 35 U.S.C. § 103(a), contrary to the Examiner's assertion.

Applicants claim a method of delivering a polypeptide into a vertebrate, comprising administering to the vertebrate a composition comprising a polynucleotide in an aqueous solution; a salt M-X dissolved in the aqueous solution at a molar concentration ranging from about 50 mM to about 250 mM, wherein M is selected from the group consisting of sodium and potassium, and X is selected from the group consisting of phosphate, acetate, bicarbonate, sulfate, and pyruvate; and an auxiliary agent selected from the group consisting of a poloxamer or reverse poloxamer; wherein chloride ion is present in the aqueous solution at a molar concentration ranging from 0 mM to about 50 mM, and wherein the polypeptide is expressed in an amount sufficient to be detectable.

Applicants note that the Examiner states that the claimed composition "also comprises a cationic lipid." (Office Action, at page 5, lines 3-4.) Contrary to the Examiner's statement, use of a transfection facilitating agent (which includes cationic lipids) is recited in only two of the currently pending claims in the application, claims 396 and 397, both of which depend either directly or indirectly from claim 358. Applicants direct the Examiner's attention to pending claim 396, which states that Applicants' claimed method *may further* comprise a transfection facilitating agent selected from the group consisting of cationic

lipids, calcium phosphate, alum, gold, tungsten, or other metal particles, transfection facilitating peptides, transfection facilitating proteins, and transfection facilitating polymers.

Liu allegedly discloses the use of poloxamers in the emulsion and micelle formulations described therein. See Liu, in Table 3, at columns 15-16; at column 17, lines 1-3; in Table 6, at column 20; and at column 20, lines 55-62, which describe polynucleotide transfection formulations containing either Pluronic® L63, Pluronic® F68, or Pluronic® F127. The presence of a poloxamer is required in Applicants' claimed polynucleotide transfection compositions, and Liu is the only document cited by the Examiner which appears to disclose polynucleotide transfection formulations containing poloxamers. The poloxamer-containing formulations disclosed in Liu, however, appear to contain either phosphate buffered saline ("PBS," containing about 150 mM NaCl and 10 mM sodium phosphate) or saline (150 mM NaCl), neither of which falls within the scope of Applicants' claimed transfection compositions. These formulations, due to the presence of 150 mM NaCl, also contain chloride ion molar concentrations that exceed the 0 mM to about 50 mM range recited in the pending amended claims. Moreover, the use of transfection compositions containing the salts recited in Applicants' amended claims at the recited concentrations, with chloride ion molar concentrations of 0 mM to about 50 mM, is not suggested in Liu. Thus, Liu does not teach or fairly suggest the use of the polynucleotide/salt/poloxamer compositions claimed by Applicants.

Liu also would not have provided the necessary motivation to one of skill in the art to combine its teachings with those of Ulmer, Wheeler, Gregoriadis, and/or Ishii, *i.e.*, to include poloxamers in the polynucleotide transfection formulations. In Liu, in Table 3, at columns 15 and 16, DNA transfection activities in BL6 and 293 cells are presented for

fifteen emulsions and micellar formulations. Only two formulations contain poloxamers, composition nos. 32 and 33, in which Pluronic® L63 is substituted for Tween 80. In comparison to the other fourteen formulations, each of these two formulations exhibit relatively low transfection activities, with eleven formulations exhibiting higher transfection activities in BL6 cells, and six formulations exhibiting higher activities in 293 cells, than the poloxamer-containing formulations. Although the transfection activities of the two Pluronic® F68 and Pluronic® F127 formulations listed in Table 6 (at column 20, in Liu) appear to be higher than those of the other formulations in the same table, they are similar to the transfection activities reported for the Brij 72 and Brij 74 formulations in Table 6. In fact, of the many examples of nonionic surfactants listed in Liu, including poloxamers (see column 7, lines 1-55), it is worth noting that the authors state that the Tween series of surfactants, rather than poloxamers, are the preferred nonionic surfactants for the disclosed invention. (See Liu, at column 7, lines 54 and 55, and at column 8, lines 26 and 27). Thus, Liu provides no motivation for including poloxamers in a transfection formulation and, in fact, may teach away from including poloxamers in such formulations.

Even if the teachings of Liu were combined with those of Ulmer, Wheeler, Gregoriadis, and/or Ishii, all the limitations of Applicants' claimed invention would not have been taught or suggested. With respect to Ulmer, Gregoriadis, and Ishii, each of these documents appears to disclose polynucleotide transfection formulations in either saline or phosphate buffered saline. Neither saline nor phosphate-buffered saline solutions contain the salts recited in Applicants' amended claims at a molar concentration falling within the recited range of about 50 mM to about 250 mM, and both saline and phosphate-buffered saline solutions also contain chloride ion molar concentrations in excess of the 0 mM to

about 50 mM concentration range recited for chloride ion in Applicants' claims. Thus, the use of transfection solutions containing the salts and molar concentrations recited in the amended claims, with chloride ion molar concentrations ranging from 0 mM to about 50 mM, is neither taught nor fairly suggested in Ulmer, Gregoriadis, or Ishii.

With respect to Wheeler, as discussed in Applicants' Amendment and Reply filed on December 23, 2002, Wheeler appears to disclose the use of a DNA transfection formulation containing 2,400 mg/L sodium bicarbonate in serum-free OPTI-MEM™ medium. Sodium bicarbonate is a salt recited in Applicants' amended claims. The molar concentration of sodium bicarbonate, however, is equivalent to 28.6 mM, which is less than the 50 mM minimum required by the amended claims. There is no suggestion in Wheeler, or in any of the other documents cited by the Examiner, to use a sodium bicarbonate concentration higher than 28 mM.

The Examiner states that "[i]t is noted that the claims require a sodium bicarbonate concentration of about 50 to about 250 mM. Although none of the cited references teaches sodium bicarbonate of this concentration, generally differences in concentration will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating that this concentration is critical." (Office Action, at page 7, lines 14-17.)

Applicants assert that differences in salt concentration, in this case, may be used to support patentability of the subject matter. Applicants direct the Examiner's attention to Example 2 of the specification, at page 81, and to Figure 2A. Example 2 and Figure 2A provide evidence that the efficiency of transfection relates to the concentration of salt in Applicants' claimed polynucleotide transfection compositions, as only sodium phosphate

concentrations of about 80 mM to about 200 mM resulted in luciferase expression levels significantly higher than those resulting from the use of saline. Accordingly, the 28.6 mM sodium bicarbonate solution in Wheeler does not teach or fairly suggest a 50 mM to 250 mM sodium bicarbonate solution. Thus, Wheeler cannot be considered, on the basis of this 28.6 mM sodium bicarbonate solution, to disclose a polynucleotide transfection composition comprising a salt at a molar concentration falling within the range required in the claimed compositions.

Moreover, because the sodium bicarbonate-containing transfection formulation disclosed in Wheeler includes serum-free OPTI-MEM™ cell culture medium, it contains a molar concentration of chloride ion greater than the concentration range required in the claimed transfection compositions. Wheeler identifies the transfection formulation as serum-free OPTI-MEM™, a modification of MEM (Eagles) medium sold by Gibco-BRL (Wheeler, at column 13, lines 37-48). The 1995-1996 Gibco-BRL product catalogue indicates that OPTI-MEM™ is a modification of Eagle's Minimum Essential Medium, buffered with HEPES and sodium bicarbonate (2.4 g/L) and supplemented with hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, and growth factors. See attached pages from the 1995-1996 Gibco-BRL product catalogue, in which the components of MEM (Eagles) medium and OPTI-MEM™ are listed. Depending on the particular formulation, MEM (Eagles) medium contains from 5.0 g/L to 8.0 g/L NaCl (commonly, about 6.8 g/L NaCl), or from at least 85 mM to at least 142 mM chloride ion (commonly, at least 116 mM chloride ion). Moreover, all MEM (Eagles) medium forumuations contain 0.4 g/L KCl, or an additional 5 mM in chloride ion concentration. Thus, the minimum molar concentration of chloride ion in any particular MEM (Eagles)

medium exceeds the 0 mM to about 50 mM chloride ion required in Applicants' claimed transfection compositions. The sodium bicarbonate-containing transfection formulation disclosed in Wheeler, therefore, neither teaches nor suggests the salts and molar concentrations, in combination with a chloride ion molar concentration that ranges from 0 mM to about 50 mM, as recited in the amended claims.

The other polynucleotide transfection formulations that appear to be disclosed in Wheeler, moreover, are either DNA/cytofectin/water solutions (see Example 3, at column 14, lines 27-29) or DNA/cytofectin/saline solutions (see Example 3, at column 15, lines 10-12, and Example 6, at column 16, lines 33-35). Neither of these formulations contains or suggests the particular salts and molar concentrations required in the claimed transfection compositions, in combination with a required chloride ion molar concentration ranging from 0 mM to about 50 mM.

Thus, the use of transfection solutions containing the salts and molar concentrations recited in Applicants' amended claims, in combination with a chloride ion molar concentration that ranges from 0 mM to about 50 mM, is neither taught nor fairly suggested in Wheeler. In fact, none of the other documents cited by the Examiner (Liu, Ulmer, Gregoriadis, and Ishii) teaches, or fairly suggests, the particular salts and molar concentrations recited in the amended claims, in combination with the recited chloride ion molar concentrations of 0 mM to about 50 mM.

Therefore, even if the teachings of Liu were combined with those of Ulmer, Wheeler, Gregoriadis, and/or Ishii, Applicants' claimed transfection compositions would not have resulted, because the resulting formulations would not have contained the salts and molar concentrations recited in the amended claims, with chloride ion molar concentrations

ranging from 0 mM to about 50 mM. Because the prior art references, when combined, must also teach or suggest all the claim limitations to establish a *prima facie* obviousness, the teachings of Liu in combination with those of Ulmer, Wheeler, Gregoriadis, and/or Ishii do not render obvious Applicants' claimed invention.

Liu, Ulmer, Gregoriadis, Ishii, and Wheeler, either alone or in combination with one another, do not teach, or fairly suggest, the use of the specific polynucleotide transfection solutions recited in the amended claims, and neither would any of these documents have suggested to one of ordinary skill in the art that the use of such solutions would be successful. Even when combined with Liu, these documents do not teach or fairly suggest all the limitations in the amended claims. Thus, none of these documents would have rendered Applicants' claimed invention *prima facie* obvious.

Applicants assert that the rejection of claims 310, 313, 314, 326, 328-334, 336, 339-343, 347-349, 315, and 352 under 35 U.S.C. § 103(a) has been overcome and respectfully request the Examiner to reconsider and withdraw this rejection.

Conclusion

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason,

that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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GIBCO BRL

Product Catalogue and Reference Guide

1995–1996

LIFE TECHNOLOGIES™

Producer of GIBCO BRL Products

OPTI-MEM® I Reduced Serum Media

OPTI-MEM I is a modification of Eagle's Minimum Essential Medium, buffered with HEPES and sodium bicarbonate (2.4 g/L) and supplemented with hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, and growth factors. The concentration of phenol red has been reduced to 1.1 mg/L. Most cells routinely cultured in serum-supplemented medium may be transferred directly into OPTI-MEM I with a minimum of 50% reduction in serum. Further significant reductions in serum requirements have been achieved with myelomas (Sp2/O-Ag14, P3×63 Ag8.653, P3-NSI-Ag4-1) and derived hybridomas, as well as with fibroblasts and epithelial cells of both normal and tumor origins. For very low serum supplementation (<1%) with attachment-dependent cells, we suggest the addition of 500 to 1,000 mg/L calcium chloride. Ideal for use during cationic lipid transfections.

Comparison of OPTI-MEM I Reduced-Serum Media.

	Base Catalogue Number 11058	21064	31985	22600	51985
with L-glutamine	●	●	●	●	
with GLUTAMAX™ I					●
with phenol red		●	●	●	●
with sodium bicarbonate	●	●	●		●
with 12.5 mM HEPES	●	●	●	●	●
with calcium chloride	●		●	●	●
liquid	●	●	●		●
powder				●	

Use this comparison table as a quick reference for identifying the base catalogue number **(in bold)** of the product that's best for your application.

OPTI-MEM I Reduced-Serum Medium (1X) liquid

Contains HEPES buffer, 2,400 mg/L sodium bicarbonate, hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, growth factors, and phenol red reduced to 1.1 mg/L.
Recommended storage condition: dark, 2°C to 8°C.
Intended use: *in vitro* diagnostic (IVD).

Cat. No.	Size	Quantity	Price
31985-021	100 ml	1–11	$ 6.00
		12–59	5.10
		60–149	4.60
31985-013	500 ml	1–11	18.90
		12–59	16.95
		60–149	15.40

OPTI-MEM I Reduced-Serum Medium (1X) liquid

Contains HEPES buffer, 2,400 mg/L sodium bicarbonate, hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, and growth factors, but no phenol red.
Recommended storage condition: dark, 2°C to 8°C.

Cat. No.	Size	Quantity	Price
11058-013	500 ml	1–11	$18.90
		12–59	16.95

OPTI-MEM I Reduced-Serum Medium (1X) liquid

Contains HEPES buffer, 2,400 mg/L sodium bicarbonate, hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, growth factors, and phenol red reduced to 1.1 mg/L, but no calcium chloride.
Recommended storage condition: dark, 2°C to 8°C.

Cat. No.	Size	Quantity	Price
21064-019	500 ml	1–11	$20.80
		12–59	18.65

OPTI-MEM I Reduced-Serum Medium (1X) liquid

A GLUTAMAX™ I medium.
Contains the dipeptide L-Alanyl-L-Glutamine substituted on a molar equivalent basis for L-glutamine.
Recommended storage condition: dark, 2°C to 8°C.

Cat. No.	Size	Quantity	Price
51985-018	100 ml	1–11	$ 6.00
		12–59	5.35
51985-026	500 ml	1–11	19.80
		12–59	17.65

OPTI-MEM I Reduced-Serum Medium, powder

A modification of MEM (Eagle's).
Contains HEPES buffer, hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, growth factors, and phenol red reduced to 1.1 mg/L, but no sodium bicarbonate.
Recommended storage condition: dark, 2°C to 8°C.
Intended use: *in vitro* diagnostic (IVD).

Cat. No.	Size	Quantity	Price
22600-050	10 × 1 L	10–90 L	$111.40
		100–290 L	102.25
		300+ L	94.76
22600-126	5 L	5–95 L	53.45
		100–295 L	49.05
		300+ L	45.50
22600-134	10 L	10–90 L	96.20
		100–290 L	87.75
		300+ L	82.00
22600-035	50 L	50–150 L	467.00
		200–250 L	411.00
		300+ L	362.40

For prices on larger quantities, please inquire.

Minimum Essential Medium[1] (MEM)

Base Catalogue No.	12570	11090	11430	11700	11095	11435	41090	51200	11890	10370
Component	1X Liquid mg/L	1X Liquid mg/L	10X Liquid mg/L	Powder mg/L	1X Liquid mg/L	10X Liquid mg/L	1X Liquid mg/L	1X Liquid mg/L	1X Liquid mg/L	1X Liquid mg/L
INORGANIC SALTS:										
$CaCl_2$ (anhyd.)	200.00	200.00	2000.00	200.00	200.00	2000.00	–	200.00	200.00	200.00
$CaCl_2 \cdot 2H_2O$	–	–	–	–	–	–	264.00	–	–	–
KCl	400.00	400.00	4000.00	400.00	400.00	4000.00	400.00	400.00	400.00	400.00
$MgSO_4$ (anhyd.)	97.67	97.67	970.67	97.67	97.67	976.70	–	97.67	97.67	97.67
$MgSO_4 \cdot 7H_2O$	–	–	–	–	–	–	200.00	–	–	–
NaCl	6800.00	6800.00	68000.00	6800.00	6800.00	68000.00	6800.00	6800.00	6800.00	6800.00
$NaHCO_3$	2200.00	2200.00	–	–	2200.00	–	2200.00	2200.00	2200.00	2200.00
$NaH_2PO_4 \cdot H_2O$[a]	140.00	140.00	1400.00	140.00	140.00	1400.00	–	140.00	140.00	140.00
$NaH_2PO_4 \cdot 2H_2O$	–	–	–	–	–	–	158.00	–	–	–
OTHER COMPONENTS:										
D-Glucose	1000.00	1000.00	10000.00	1000.00	1000.00	10000.00	1000.00	1000.00	1000.00	1000.00
Phenol Red	10.00	10.00	100.00	6.00	10.00	100.00	10.00	–	10.00	10.00
Sodium Succinate	–	–	–	100.00	–	–	–	–	–	–
Succinic Acid	–	–	–	75.00	–	–	–	–	–	–
AMINO ACIDS:										
L-Alanine	–	–	–	–	–	–	–	–	–	9.00
L-Arginine • HCl	126.00	126.00	1260.00	126.00	126.00	1260.00	126.00	126.00	126.00	126.00
L-Asparagine•H_2O	–	–	–	–	–	–	–	–	–	13.00
L-Cystine	–	–	–	–	–	–	24.00	–	–	–
L-Cystine • 2HCl	31.00	31.00	–	31.00	31.00	–	–	31.00	31.00	31.00
L-Cystine • 2Na	–	–	286.00	–	–	286.00	–	–	–	–
L-Glutamic acid	–	–	–	–	–	–	–	–	–	15.00
L-Glutamine	292.00	–	–	–	292.00	2920.00	–	–	–	–
L-Alanyl-L-Glutamine	–	–	–	–	–	–	406.00	–	–	–
Glycine	–	–	–	–	–	–	–	–	–	8.00
L-Histidine HCl • H_2O	42.00	42.00	420.00	42.00	42.00	420.00	42.00	42.00	42.00	42.00
L-Isoleucine	52.00	52.00	520.00	52.00	52.00	520.00	52.00	52.00	52.00	52.00
L-Leucine	52.00	52.00	520.00	52.00	52.00	520.00	52.00	52.00	–	52.00
L-Lysine • HCl	73.00	73.00	730.00	73.00	73.00	730.00	73.00	73.00	73.00	72.50
L-Methionine	15.00	15.00	150.00	15.00	15.00	150.00	15.00	15.00	15.00	15.00
L-Phenylalanine	32.00	32.00	320.00	32.00	32.00	320.00	32.00	32.00	32.00	32.00
L-Proline	–	–	–	–	–	–	–	–	–	12.00
L-Serine	–	–	–	–	–	–	–	–	–	11.00
L-Threonine	48.00	48.00	480.00	48.00	48.00	480.00	48.00	48.00	48.00	48.00
L-Tryptophan	10.00	10.00	100.00	10.00	10.00	100.00	10.00	10.00	10.00	10.00
L-Tyrosine	–	–	–	36.00	–	–	36.00	–	–	–
L-Tyrosine • 2Na • $2H_2O$	52.00	52.00	520.00	–	52.00	520.00	–	52.00	52.00	52.00
D-Valine	92.00	–	–	–	–	–	–	–	–	–
L-Valine	–	46.00	460.00	46.00	46.00	460.00	46.00	46.00	46.00	46.00
VITAMINS:										
D-Ca Pantothenate	1.00	1.00	10.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
Choline Bitartrate	–	–	–	1.80	–	–	–	–	–	–
Choline Chloride	1.00	1.00	10.00	–	1.00	10.00	1.00	1.00	1.00	1.00
Folic Acid	1.00	1.00	10.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
i-Inositol	2.00	2.00	20.00	2.00	2.00	20.00	2.00	2.00	2.00	2.00
Niacinamide	1.00	1.00	10.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
Pyridoxal HCl	1.00	1.00	10.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
Riboflavin	0.10	0.10	1.00	0.10	0.10	1.00	0.10	0.10	0.10	0.10
Thiamine HCl	1.00	1.00	10.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00

1. Eagle, H. (1959) *Science, 130,* 432.
a. Original formula lists this component as $NaH_2PO_4 \cdot 2H_2O$.

Minimum Essential Medium[1] (MEM)

Base Catalogue No.	61100	12360	32400	41500	11570	11581	42360	12380	11585	21097	11112
Component	Powder mg/L	1X Liquid mg/L	Powder mg/L	Powder mg/L	1X Liquid mg/L	10X Liquid mg/L	1X Liquid mg/L	1X Liquid mg/L	10X Liquid mg/L	1X Liquid mg/L	1X Liquid mg/L
INORGANIC SALTS:											
$CaCl_2$ (anhyd.)	200.00	200.00	200.00	200.00	140.00	1400.00	–	–	1400.00	200.00	200.00
$CaCl_2 \cdot 2H_2O$	–	–	–	–	–	–	264.00	–	–	–	–
KCl	400.00	400.00	400.00	400.00	400.00	4000.00	400.00	400.0	4000.00	400.00	400.00
KH_2PO_4	–	–	–	–	60.00	600.00	–	–	600.00	–	–
$MgCL_2 \cdot 6H_2O$	–	–	–	–	–	1000.00	–	–	1000.00	–	–
$MgSO_4$ (anhyd.)	97.67	97.67	97.67	97.67	97.67	–	–	97.67	–	97.67	97.67
$MgSO_4 \cdot 7H_2O$	–	–	–	–	–	1000.00	200.00	–	1000.00	–	–
NaCl	6800.00	6300.00	6800.00	6800.00	8000.00	80000.00	6300.00	5000.00	80000.00	6800.00	6800.00
$NaHCO_3$	–	2200.00	–	–	350.00	–	2200.00	2200.00	–	2200.00	2200.00
$NaH_2PO_4 \cdot H_2O$[a]	140.00	140.00	140.00	140.00	–	–	–	1400.00	–	–	140.00
$NaH_2PO_4 \cdot 2H_2O$	–	–	–	–	–	–	158.00	–	–	–	–
$Na_2HPO_4 \cdot 7H_2O$	–	–	–	–	–	900.00	–	–	900.00	–	–
Na_2HPO_4	–	–	–	–	48.00	–	–	–	–	–	–
OTHER COMPONENTS:											
D-Glucose	1000.00	1000.00	1000.00	1000.00	1000.00	10000.00	1000.00	1000.00	10000.00	1000.00	1000.00
HEPES	–	5958.00	–	–	–	–	5960.00	5958.00	–	–	–
Phenol Red	10.00	10.00	10.00	10.00	10.00	100.00	10.00	10.00	100.00	10.00	10.00
AMINO ACIDS:											
L-Alanine	–	–	–	8.90	–	–	–	–	–	–	–
L-Arginine • HCl	126.00	126.00	126.00	126.00	126.00	1260.00	126.40	126.00	1260.00	126.00	126.00
L-Asparagine • H_2O	–	–	–	13.20	–	–	–	–	–	–	–
L-Aspartic Acid	–	–	–	13.30	–	–	–	–	–	–	–
L-Cystine	–	–	–	–	–	240.00	24.02	–	240.00	–	–
L-Cystine • 2HCl	31.00	31.00	31.00	31.00	31.00	–	–	31.00	–	31.00	31.00
L-Glutamic Acid	–	–	–	14.70	–	–	–	–	–	–	–
L-Glutamine	292.00	–	292.00	292.00	–	–	–	–	2920.00	292.00	–
L-Alanyl-L-Glutamine	–	–	–	–	–	–	434.00	–	–	–	–
Glycine	–	–	–	7.50	–	–	–	–	–	–	–
L-Histidine HCl • H_2O	42.00	42.00	42.00	42.00	42.00	420.00	41.92	42.00	420.00	42.00	42.00
L-Isoleucine	52.00	52.00	52.00	52.00	52.00	520.00	52.46	52.00	520.00	52.00	52.00
L-Leucine	52.00	52.00	–	52.00	52.00	520.00	52.46	52.00	520.00	52.00	52.00
L-Lysine • HCl	73.00	73.00	–	72.50	73.00	730.00	73.06	73.00	730.00	73.00	73.00
L-Methionine	15.00	15.00	–	15.00	15.00	150.00	14.92	15.00	150.00	15.00	15.00
L-Phenylalanine	32.00	32.00	32.00	32.00	32.00	320.00	33.02	32.00	320.00	32.00	32.00
L-Proline	–	–	–	11.50	–	–	–	–	–	–	–
L-Serine	–	–	–	10.50	–	–	–	–	–	–	–
L-Threonine	48.00	48.00	48.00	48.00	48.00	480.00	47.64	48.00	480.00	48.00	48.00
L-Tryptophan	10.00	10.00	10.00	10.00	10.00	100.00	10.20	10.00	100.00	10.00	10.00
L-Tyrosine	–	–	–	–	–	360.00	36.22	–	360.00	–	–
L-Tyrosine (disodium salt)	52.00	52.00	52.00	52.00	52.00	–	–	52.00	–	52.00	52.00
L-Valine	46.00	46.00	46.00	46.00	46.00	460.00	46.86	46.00	460.00	46.00	46.00
VITAMINS:											
D-Ca Pantothenate	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	10.00	1.00	1.00
Choline Chloride	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	10.00	1.00	1.00
Folic Acid	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	10.00	1.00	1.00
i-Inositol	2.00	2.00	2.00	2.00	2.00	20.00	2.00	2.00	20.00	2.00	2.00
Niacinamide	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	10.00	1.00	1.00
Pyridoxal HCl	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	10.00	1.00	1.00
Riboflavin	0.10	0.10	0.10	0.10	0.10	1.00	0.10	0.10	1.00	0.10	0.10
Thiamine HCl	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	10.00	1.00	1.00
FBS	–	–	–	–	–	–	–	–	–	–	100 ml

1. Eagle, H. (1959) *Science, 130,* 432.
a. Original formula lists this component as $NaH_2PO_4 \cdot 2H_2O$.

Minimum Essential Medium[1] (MEM)

Catalogue No. / Component	11575 1X Liquid mg/L	41200 Powder mg/L	12370 1X Liquid mg/L	22500 Powder mg/L	41600 Powder mg/L	11380 1X Liquid mg/L	11650 10X Liquid mg/L	21800 Powder mg/L	11385 1X Liquid mg/L	11400 Powder mg/L	22300 Powder mg/L
INORGANIC SALTS:											
$CaCl_2$ (anhyd.)	140.00	140.00	140.00	200.00	140.00	–	–	–	–	–	–
$CaCl_2 \cdot 2H_2O$	–	–	–	–	–	–	–	–	–	–	–
KCl	400.00	400.00	400.00	400.00	400.00	400.00	4000.00	400.00	400.00	400.00	400.00
KH_2PO_4	60.00	60.00	60.00	60.00	60.00	–	–	–	–	–	–
$MgCl_2$ (anhyd.)	–	–	–	–	–	–	–	–	–	–	94.70
$MgCl_2 \cdot 6H_2O$	–	–	–	–	–	–	–	–	–	–	–
$MgSO_4$ (anhyd.)	97.67	97.67	97.67	97.67	97.67	97.67	–	97.67	97.67	97.67	–
$MgSO_4 \cdot 7H_2O$	–	–	–	–	–	–	2000.00	–	–	–	–
NaCl	8000.00	8000.00	6800.00	6800.00	8000.00	6800.00	68000.00	6800.00	6800.00	6800.00	6500.00
$NaHCO_3$	350.00	–	350.00	–	–	2200.00	–	–	2200.00	–	–
$NaH_2PO_4 \cdot H_2O$[a]	–	–	–	–	–	1400.00	14,000.00	1400.00	1400.00	1400.00	1340.00
$NaH_2PO_4 \cdot 2H_2O$	–	–	–	–		–	–	–	–	–	–
Na_2HPO_4 (anhyd.)	48.00	48.00	48.00	–	48.00	–	–	–	–	–	–
OTHER COMPONENTS:											
D-Glucose	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	10000.00	1000.00	1000.00	1000.00	2000.00
HEPES	–	–	5958.00	–	–	–	–	–	–	–	–
Phenol Red	10.00	10.00	10.00	10.00	10.00	10.00	100.00	6.00	10.00	10.00	10.00
Sodium Succinate	–	–	–	–	–	–	–	100.00	–	–	–
Succinic Acid	–	–	–	–	–	–	–	75.00	–	–	–
AMINO ACIDS:											
L-Alanine	–	–	–	–	8.90	–	–	–	–	–	–
L-Arginine • HCl	126.00	126.00	126.00	126.00	126.00	126.00	1260.00	126.00	126.00	126.00	126.00
L-Asparagine	–	–	–	–	13.20	–	–	–	–	–	–
L-Aspartic Acid	–	–	–	–	13.30	–	–	–	–	–	–
L-Cystine	–	–	–	–	–	–	240.00	–	–	–	–
L-Cystine • 2HCl	31.00	31.00	31.00	31.00	31.00	31.00	–	31.00	31.00	31.00	31.00
L-Glutamic Acid	–	–	–	–	14.70	–	–	–	–	–	–
L-Glutamine	292.00	292.00	–	292.00	292.00	–	–	–	292.00	292.00	292.00
Glycine	–	–	–	–	7.50	–	–	–	–	–	–
L-Histidine	–	–	–	–	–	–	–	–	–	–	31.00
L-Histidine HCl • H_2O	42.00	42.00	42.00	42.00	42.00	42.00	420.00	42.00	42.00	42.00	–
L-Isoleucine	52.00	52.00	52.00	52.00	52.00	52.00	520.00	52.00	52.00	52.00	52.00
L-Leucine	52.00	52.00	52.00	52.00	52.00	52.00	520.00	52.00	52.00	52.00	52.00
L-Lysine	–	–	–	–	–	–	–	–	–	–	58.00
L-Lysine • HCl	73.00	73.00	73.00	73.00	73.00	73.00	730.00	73.00	73.00	73.00	–
L-Methionine	15.00	15.00	15.00	15.00	15.00	15.00	150.00	15.00	15.00	15.00	15.00
L-Phenylalanine	32.00	32.00	32.00	32.00	32.00	32.00	320.00	32.00	32.00	32.00	32.00
L-Proline	–	–	–	–	11.50	–	–	–	–	–	–
L-Serine	–	–	–	–	10.50	–	–	–	–	–	–
L-Threonine	48.00	48.00	48.00	48.00	48.00	48.00	480.00	48.00	48.00	48.00	48.00
L-Tryptophan	10.00	10.00	10.00	10.00	10.00	10.00	100.00	10.00	10.00	10.00	10.00
L-Tyrosine	–	–	–	–	–	–	360.00	36.00	–	–	–
L-Tyrosine (disodium salt)	52.00	52.00	52.00	52.00	52.00	52.00	–	–	52.00	52.00	52.00
L-Valine	46.00	46.00	46.00	46.00	46.00	46.00	460.00	46.00	46.00	46.00	46.00
VITAMINS:											
D-Ca Pantothenate	1.00	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
Choline Bitartrate	–	–	–	–	–	–	–	1.80	–	–	–
Choline Chloride	1.00	1.00	1.00	1.00	1.00	1.00	10.00	–	1.00	1.00	1.00
Folic Acid	1.00	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
i-Inositol	2.00	2.00	2.00	2.00	2.00	2.00	20.00	2.00	2.00	2.00	2.00
Niacinamide	1.00	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
Pyridoxal HCl	1.00	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
Riboflavin	0.10	0.10	0.10	0.10	0.10	0.10	1.00	0.10	0.10	0.10	0.10
Thiamine HCl	1.00	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00

1. Eagle, H. (1959) *Science, 130,* 432.
a. Original formula lists this component as $NaH_2PO_4 \cdot 2H_2O$.